

FIGURE 1

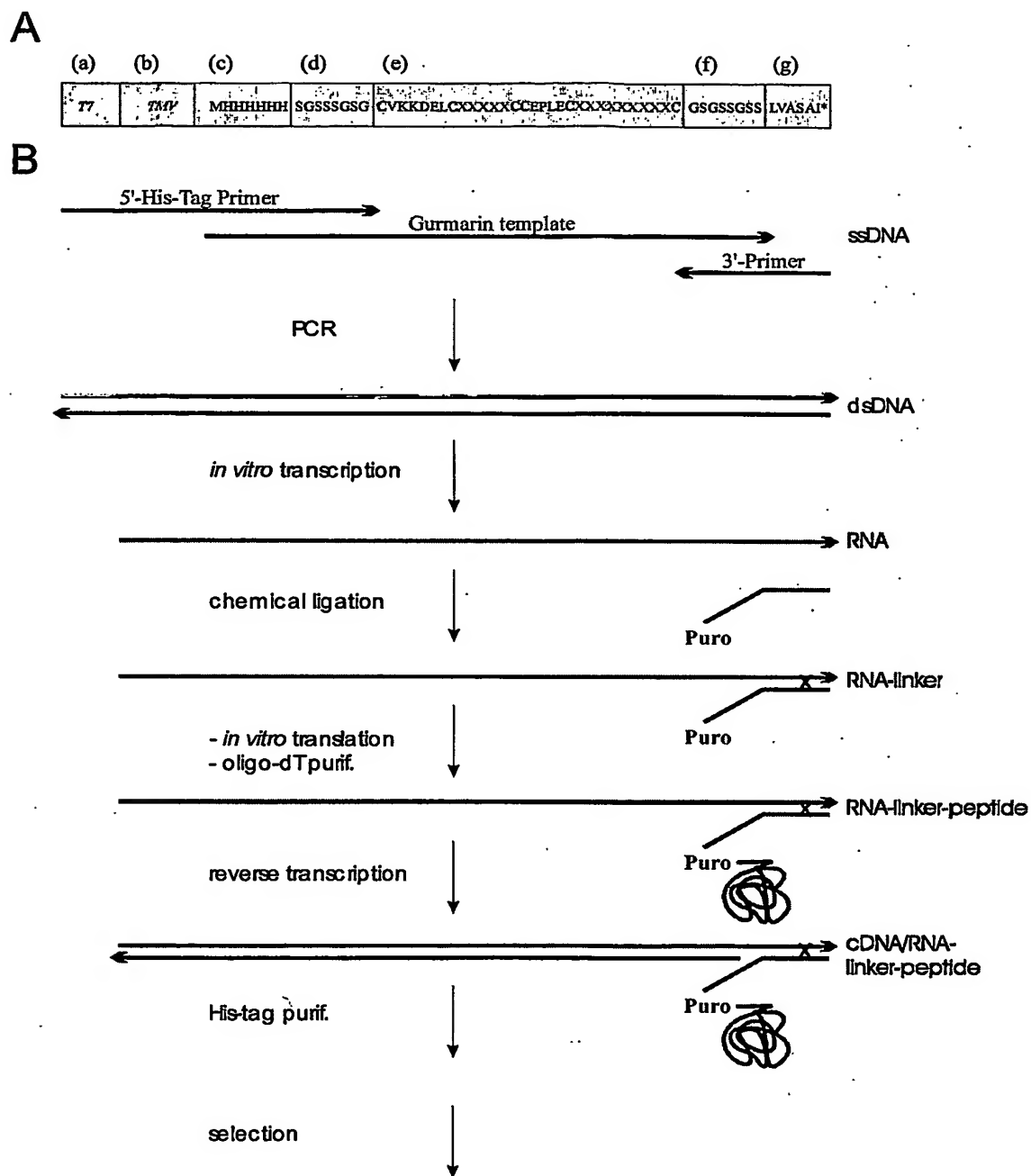
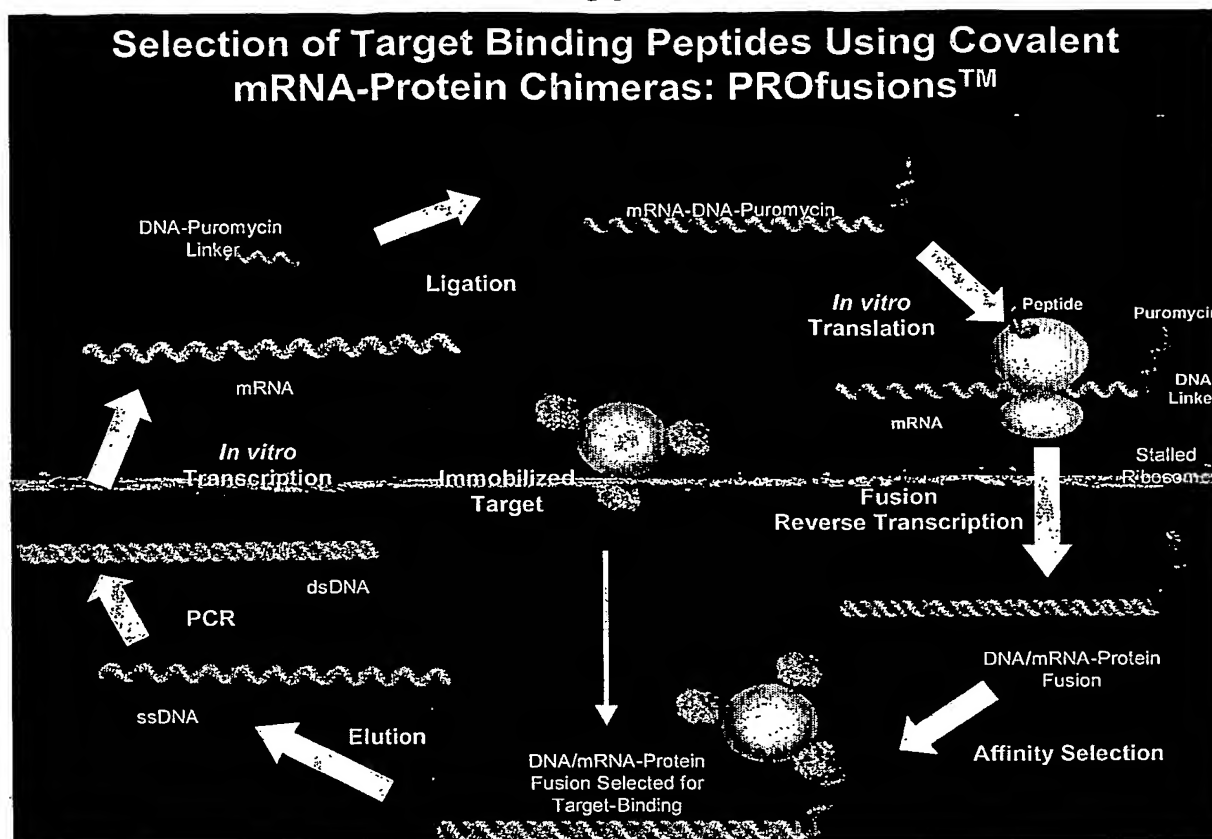
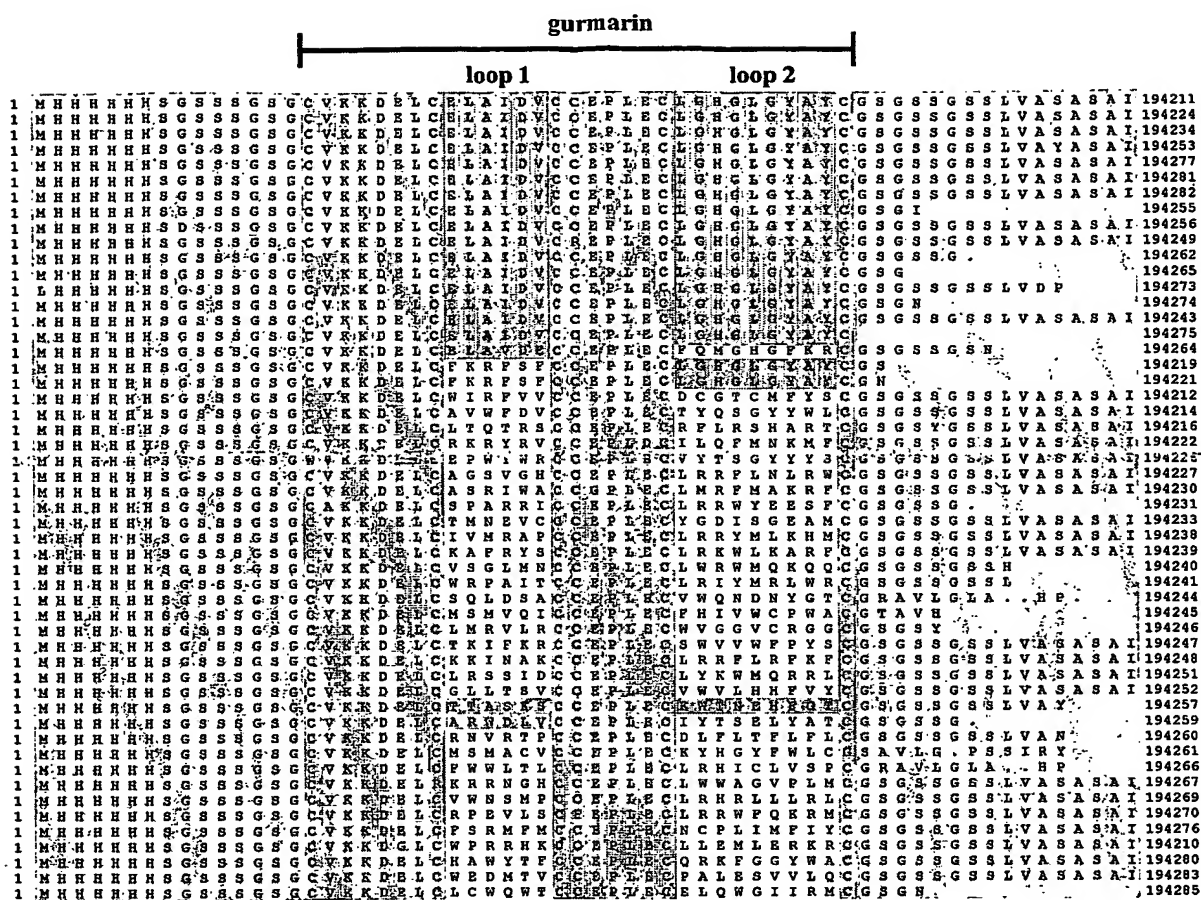


FIGURE 2

Target: Pertussis toxin		Library: Gurmarin							
		R1	R2	R3	R4	Epoxy-PT R5a	biot PT R5b	biot PT R6a	R6b
								from R5a	from R5b
1.	PCR								
1.1.	Analytical RT-PCR								
	specific signal after x rounds of PCR		18	24	24	24		24	15
	control signal after y rounds of PCR		21	27	24	27		27	24
1.2.	Preparative PCR								
	done with z rounds of PCR		36	30	30	32		30	30
2.	In vitro transcription								
	scale		2x 500 µl	1x 500 µl	1x 500 µl	1x 500 µl		1x 500 µl	1x 500 µl
	DNase digestion done		no	yes	yes	yes		yes	yes
3.	NAP5 gel filtration								
	Input		500/300/400/200	500/300/400/200	500/300/400/200	500/300/400/200		500/300/400/200	500/300/400/200
	yield purified RNA in pooled E1 (QD260)		20 nmol	13 nmol	12.7 nmol	11.6 nmol		16.3 nmol	17.6 nmol
	remaining material					6.6 nmol			
4.	Linker coupling								
	Linker type		PEG2A18	PEG2	PEG2	PEG2		PEG2	PEG2
	Input		6 nmol	5 nmol	5 nmol	5 nmol		5 nmol	5 nmol
	coupling efficiency		70%	70%	70%	70%		70%	70%
	yield linker coupled RNA		4.2 nmol	3.5 nmol	3.5 nmol	3.5 nmol		3.5 nmol	3.5 nmol
6.	In vitro Translation and PROFusion™								
	formation								
	Input		4.2 nmol	3.5 nmol	3 nmol	3 nmol		3 nmol	3 nmol
	RNA / 200 µl lysate		260 pmol	290 pmol	250 pmol	250 pmol		250 pmol	250 pmol
	salt incubation at -20°C		over night	over night	over night	over night		over night	over night
6.	Oligo(dT) purification								
	purified on x columns		?	4	4	4		4	4
	efficiency		4.5%	4.4%	2.7%	7.2%		3.3%	2.7%
	yield		187 pmol	156 pmol	81.1 pmol	217 pmol		97.6 pmol	81.1 pmol
7.	Reverse transcription								
	conditions		1 mM DTT	1 mM DTT	1 mM DTT	1 mM DTT		1 mM DTT	1 mM DTT
	Input		168 pmol (3/4)	72.6 pmol (1/2)	50.4 pmol (3/4)	50 pmol		40 pmol	50 pmol
8.	His-tag purification								
	Input		168 pmol	90.75 pmol (72 pmol + 18 pmol ohne RT)	61.9 pmol (3/4 RT + 1/4 OdT)	45 pmol RT + 27.6 pmol RNA		35 pmol RT + 15 pmol	43 pmol RT + 19 pmol
	efficiency		34%	40%	43%	41%		36%	43%
	yield		55 pmol	66.6 pmol	26.4 pmol	27.6 pmol		18 pmol	26.4 pmol
	In volume of		450 µl	450 µl	450 µl	450 µl		450 µl	450 µl
9.	selection								
	selection volume		1 ml	1 ml	1 ml	1 ml		1 ml	1 ml
	Input		18 pmol	5 pmol	5 pmol	5 pmol		5 pmol	5 pmol
	final concentration of Imidazol		45 mM	6 mM	6 mM	11.4 mM		18.8 mM	21.5 mM
	preclear		3x 100 µl HBS blocked streptavidin beads	3x 100 µl HBS blocked epoxy beads	3x 100 µl HBS blocked, biotin saturated streptavidin beads	2x 100 µl HBS blocked epoxy beads	3x 100 µl HBS blocked, biotin saturated streptavidin beads	3x 100 µl HBS blocked, biotin saturated streptavidin beads	3x 100 µl HBS blocked, biotin saturated streptavidin beads
	first pre-clear binding		0%	background	background	23/24 dpm	80/50/32 dpm	37/33/34 dpm	72/30/37 dpm
	beads saturated with a pulse of biotin		yes	yes	yes	no	yes	yes	yes
	effective concentration of PT		100 nM (50 nM)	100 nM (50 nM)	100 nM (50 nM)	50 nM	100 nM (50 nM)	100 nM (50 nM)	100 nM (50 nM)
	conditions				60 min at RT	90 min at RT	90 min at RT	90 min at RT	90 min at RT
	specific binding %		-	-	0.2%	1.0%	5.2%	9.4%	11.6%

FIGURE 4



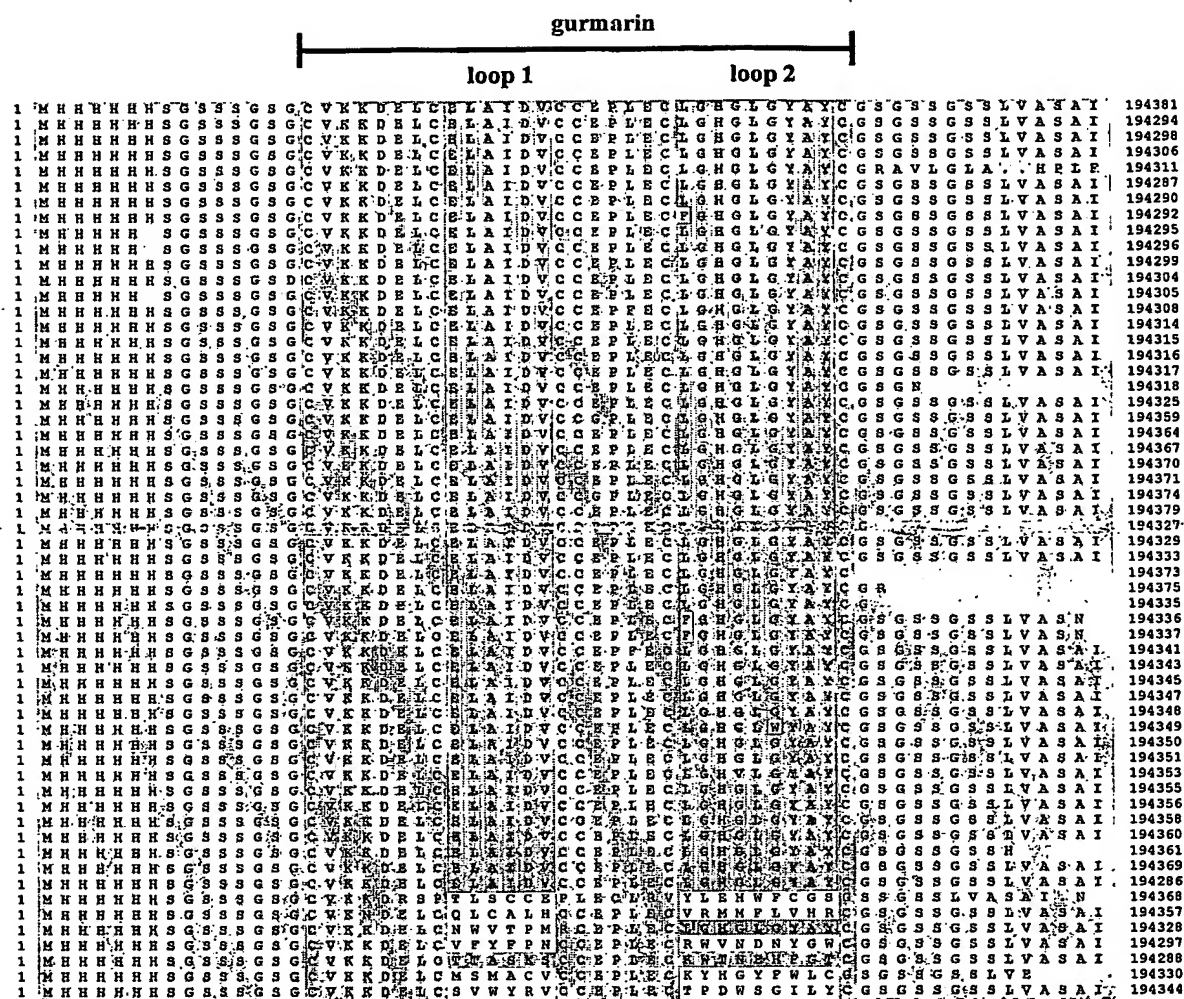


FIGURE 5

gurmarin

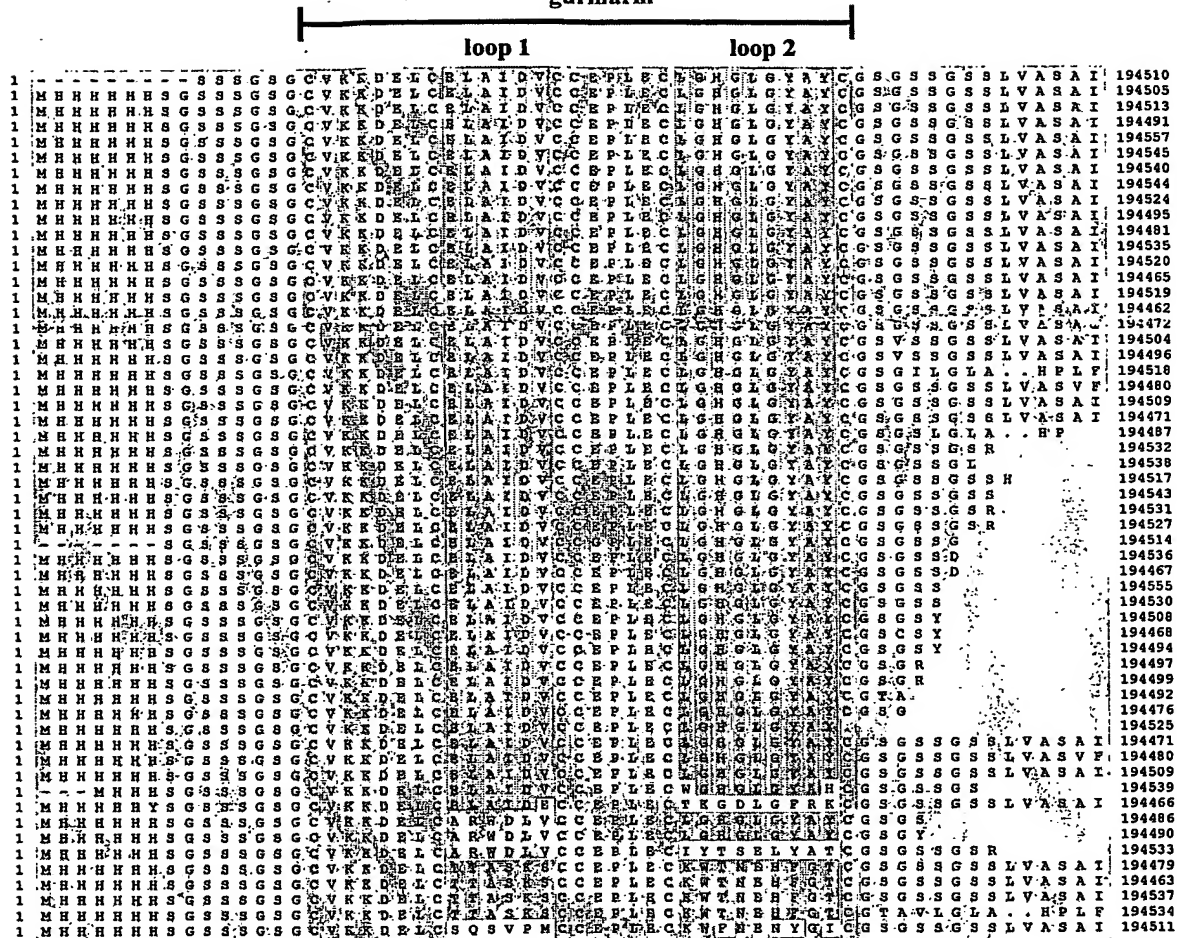


FIGURE 8

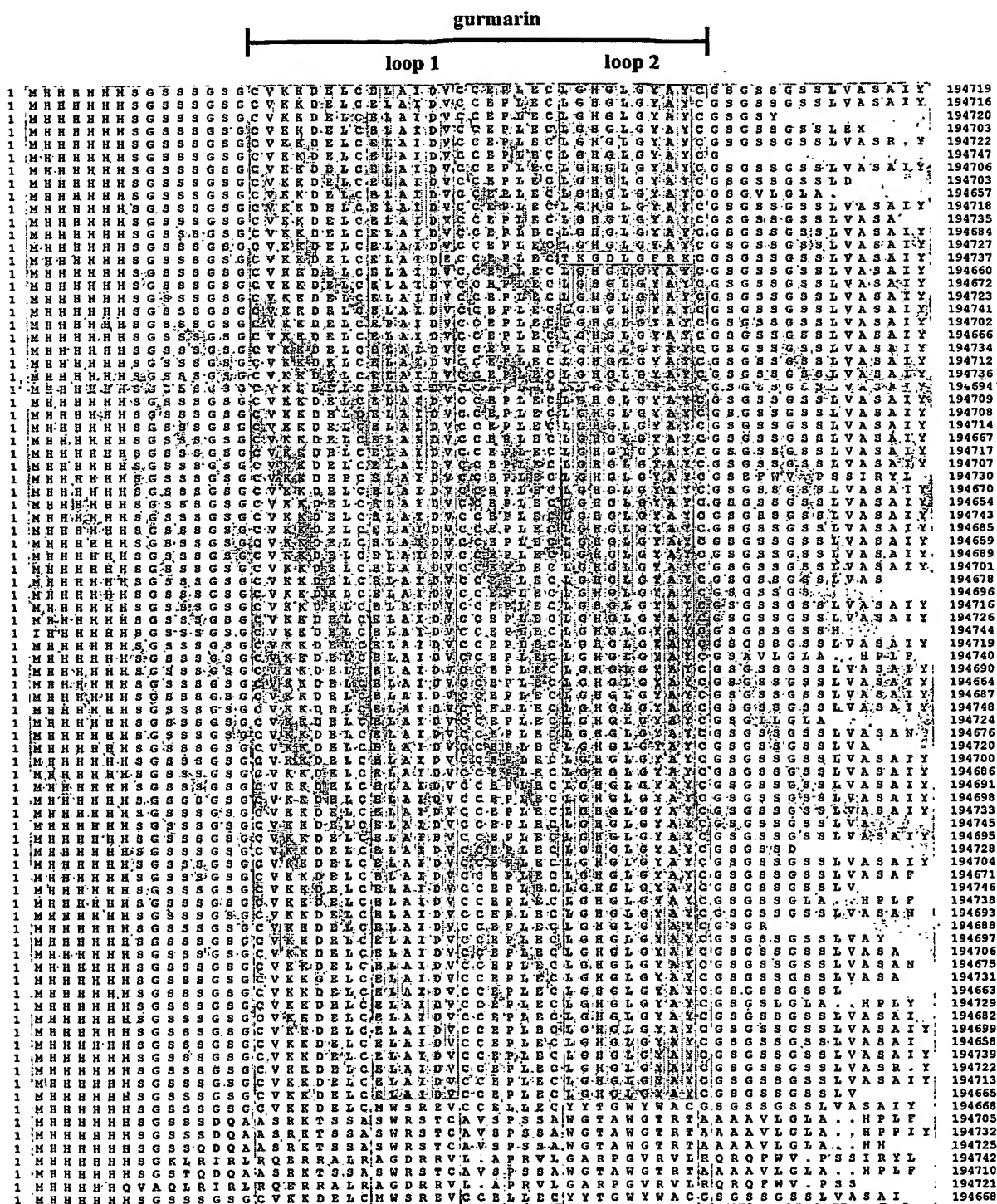


Table 2: selection overview of PB26 selection against immobilized PT

Library: PP26									
Target: Pertussis toxin									
		R1	R2	R3	R4	epoxy bead Imm. PT	biot PT	biot PT	biot PT
	PCR						R6a	R6b	R6b
1.1.	Analytical RT-PCR								
	specific signal after x rounds of PCR		18	18	24	24	24		
	control signal after y rounds of PCR		21	24	24	24	24		
1.2.	Preparative PCR								
	done with z rounds of PCR		36	30	30	32			
	quality			single band	single band	single band			
2.	In vitro transcription								
	scale		2x 500 µ	1x 500 µ	1x 500 µ	1x 500 µ			
	DNase digestion done		no	no	yes	yes			
	quality								
3.	NAPs gel filtration								
	Input/E1/E2/E3		500/500/400/200	500/500/400/200	500/500/400/200	500/500/400/200			
	yield purified RNA in pooled E2 (OD280)		28 nmol	16 nmol	6.3 nmol	12.4 nmol			
4.	Linker coupling								
	Linker type		PEG2	PEG2	PEG2	PEG2			
	Input		6 nmol	4.5 nmol	5 nmol	5 nmol			
	coupling efficiency		70%	70%	70%	70%			
	yield linker coupled RNA		4.2 nmol	3.15 nmol	3.5 nmol	3.5 nmol			
	quality								
6.	In vitro Translation and Fuzagen formation								
	Input		4.2 nmol	3.15 nmol	3 nmol	3 nmol			
	RNA/200 µ		260 pmol	260 pmol	250 pmol	250 pmol			
	cell incubation at -20C		over weekend	over night	over night	over night			
6.	Oligo(dT) purification								
	purified on x columns		?	4	4	4			
	efficiency		8%	1.89%	2.1%	5.8%			
	yield		68 pmol	56.7 pmol	64.9 pmol	175 pmol			
7.	Reverse transcription								
	conditions		1 mM DTT	1 mM DTT	1 mM DTT	1 mM DTT			
	Input		68 pmol (3/4)	42.5 pmol (3/4)	40.3 pmol (3/4)	50 pmol			
	efficiency of RT								
	yield of RT-RNA								
	portion of reverse transcribed Fuzagens								
8.	His-Tag purification on Ni-NTA agarose								
	conditions: endconcentration of DTT from RT		0.75 mM	0.7 mM	0.52 mM	0.5 mM			
	Input		68 pmol	42.5 pmol (RT Ansatz, ohne nicht-RT)	47 pmol (3/4 RT + 1/4 CoT)	45 pmol RT + 21 pmol RNA			
	efficiency		34%	30.6%	35%	41%			
	yield		55 pmol (100%)	19.7 pmol	18.5 pmol	27.8 pmol			
	In volume of		450 µ	300 µ	450 µ	450 µ			
	Oligo(dT) purification								
9.	selection								
	selection volume		1 ml	1 ml	1 ml	1 ml			
	Input		18 pmol	5 pmol	5 pmol	5 pmol			
	final concentration of Imidazole		25 mM	18 mM	19.5 mM	12.3 mM			
	predear		5x 100 µ streptavidin beads	5x 100 µ streptavidin beads	3x 100 µ epoxy beads	2x 100 µ blocked epoxy beads			
	first pre-clear binding %		0%	0.5%	background	42 dpm			
	selection on biotinylated Pertussis toxin (100%)		0%	0.5%	0.5%	0.3%			
	Epoxy-bead coupled PT		serie 1	serie 1	serie 1	serie 1			
	PT- beads saturated with a pulse of biotin		yes	yes	yes	no			
	effective concentration of PT		50 nM (100%)	50 nM (100%)	50 nM (100%)	100 nM (50 nM)			
	conditions		over night at 4C	60 min at RT	60 min at RT	90 min at RT			
	specific binding %				1.2%	0.5%			
						2.8%			
						3.5%			

197588 MGRGSHHHHHHARS NVIPLNEVWYDTGWDREHRSRLSTDD DANAPKASAI
197947 MGRGSHHHHHHARS VGTIRIAQDTEHYRNVRHKLSQLSR DANAPKASAI

198000 MGRGSHHHHHHARS WRDTRKLLHMRHYFPLAIDSYWDHTLR DANAPKASAI
197983 MGRGSHHHHHHARS ETSMDGCELRTRDDELTATTKHSSHPF DANAPKASAI
197998 MGRGSHHHHHHARS SPLWYHNCWD TICLADWLKDRPHGVY DANAPKASA

197836 MGRGSHHHHHHARS [REDACTED] DANAPKASAI
197552 MGRGSHHHHHHARS [REDACTED] DANAPKASAI

197596 MGRGSHHHHHHARS TMTNTHMLDRLMTNHVKRDS SPGSI DANAPKASAI

197569 MGRGSHHHHHHARS DWELSPPHVAITTRHLINCTDGP LLRDANAPKASAI
197536 MGRGSHHHHHHARS LNGESTSNILTTSRKVTEWTGYTASVDANAPKASAI

197611 MGRGSHHHHHHARS QVTWHHLADTVTTKNRKCTDSYIGWNXANAPKASAI
197530 MGRGSHHHHHHARS IIVIHNAIQHTTPHQVSIWCPPKHNRDANAPKASAI
197557 MGRGSHHHHHHARS SHCRHRNCHTITRGNMRIETFPNNIRK DANAPKASAI

197797 MGRGSHHHHHHARS SWGLSGTQTWKITKLATRLHHPEFETNDANAPKASAI

197888 MGRGSHHHHHHARS WRWHNWGLSDTVASHPDASNSLNMMY DANAPKASAN
197897 MGRGSHHHHHHLDLWGPPSGSPRTRSTTGTSTTSSPSTPGTITLRRHPH
197825 MGRGSHHHHHHARS SWQPEVKMSSLVDTSQTVGA AVETRRTT DANAPKASA

FIGURE 9

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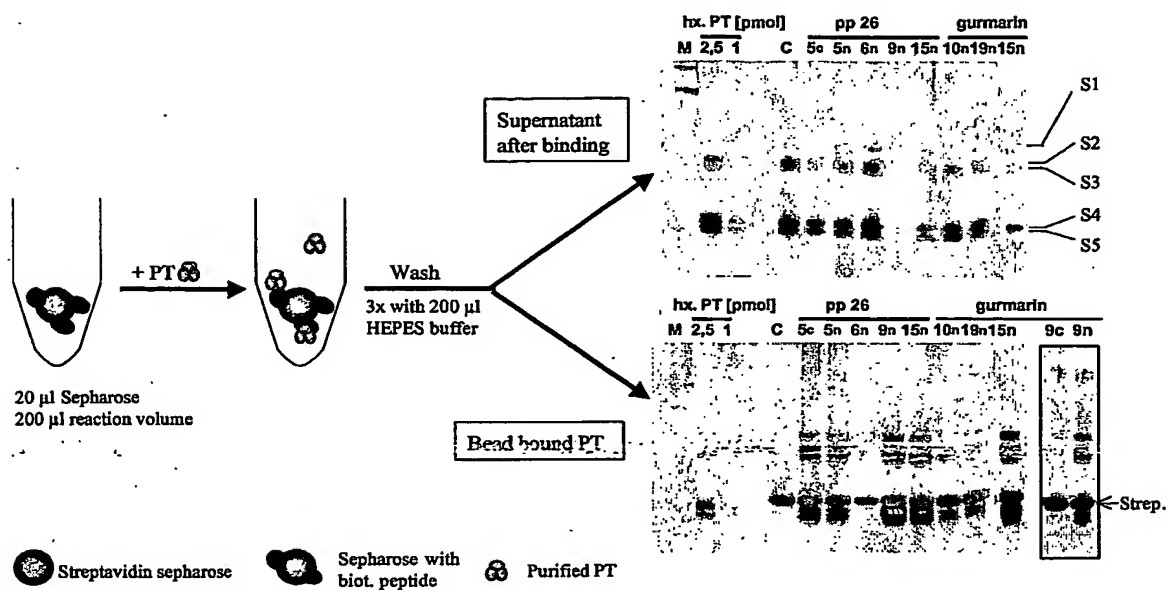
FIGURE 10

1 MGRGSHHHHHHARSNVIPTLHVHYDNGDRERHSLSTDDANAPKASAI 197588
1 MGRGSHHHHHHARSNVIPTLHVHYDNGDRERHSLSTDDANAPKASAI 197576
1 MGRGSHHHHHHARSNVIPTLHVHYDNGDRERHSLSTDDANAPKASAI 197615
1 MGRGSHHHHHHARSNVIPTLHVHYDNGDRERHSLSTDDANAPKASAI 197585
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1 MGRGSHHHHHHARSNVIPTLHVHYDNGDRERHSLSTDDANAPKASAI 197592
1 MGRGSHHHHHHARSNVIPTLHVHYDNGDRERHSLSTDDANAPKASAI 197596

FIGURE 14

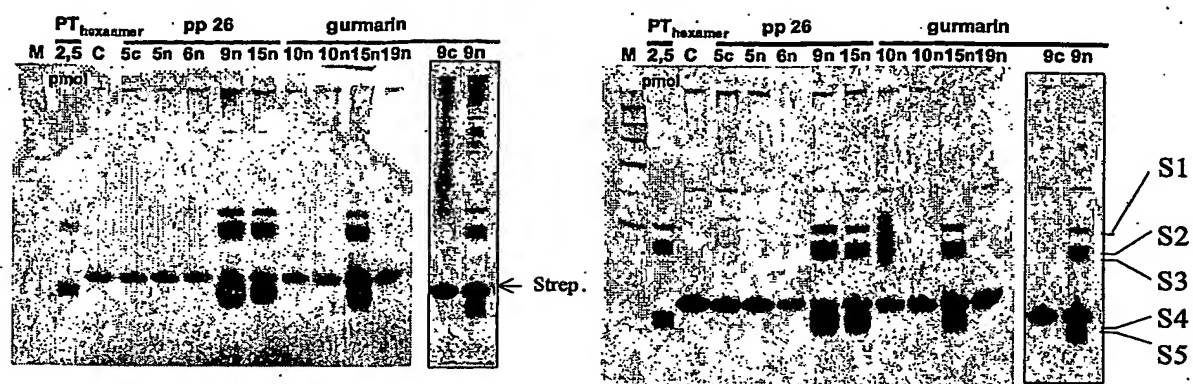
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FIGURE 15



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FIGURE 16



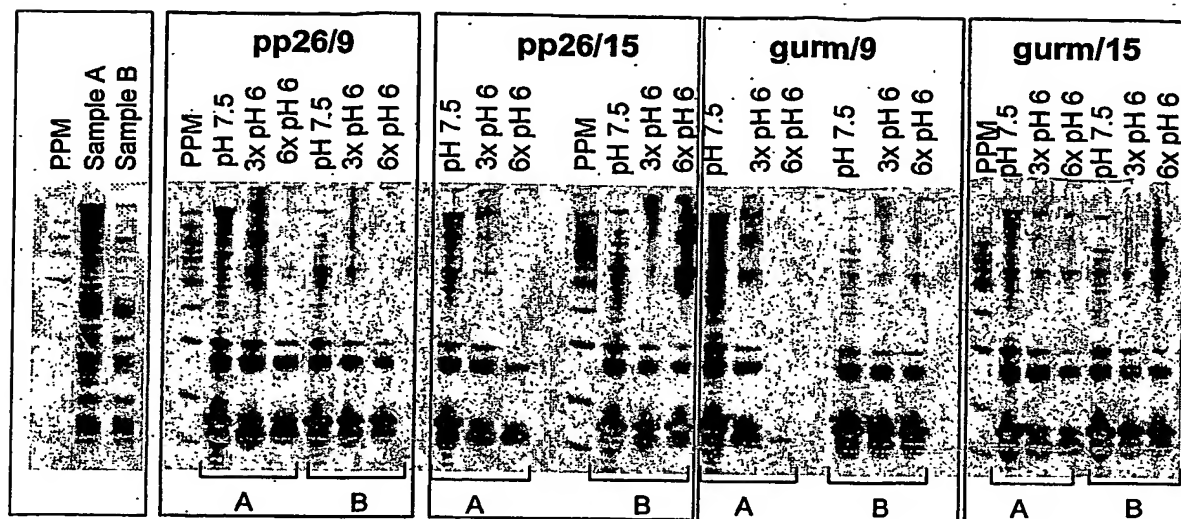
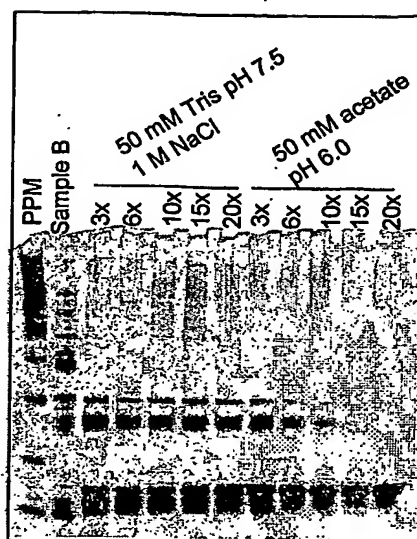
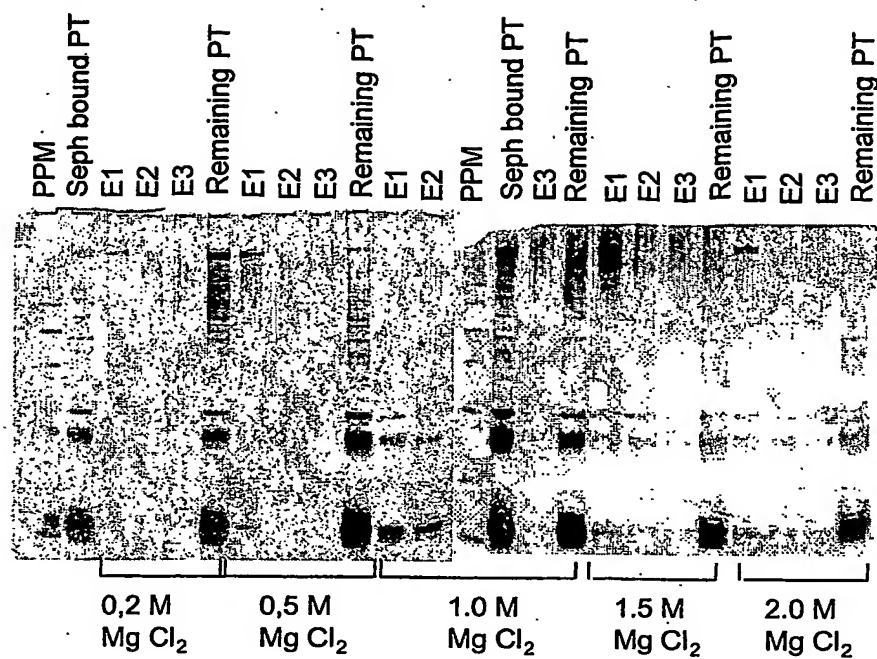
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FIGURE 17

FIGURE 18.

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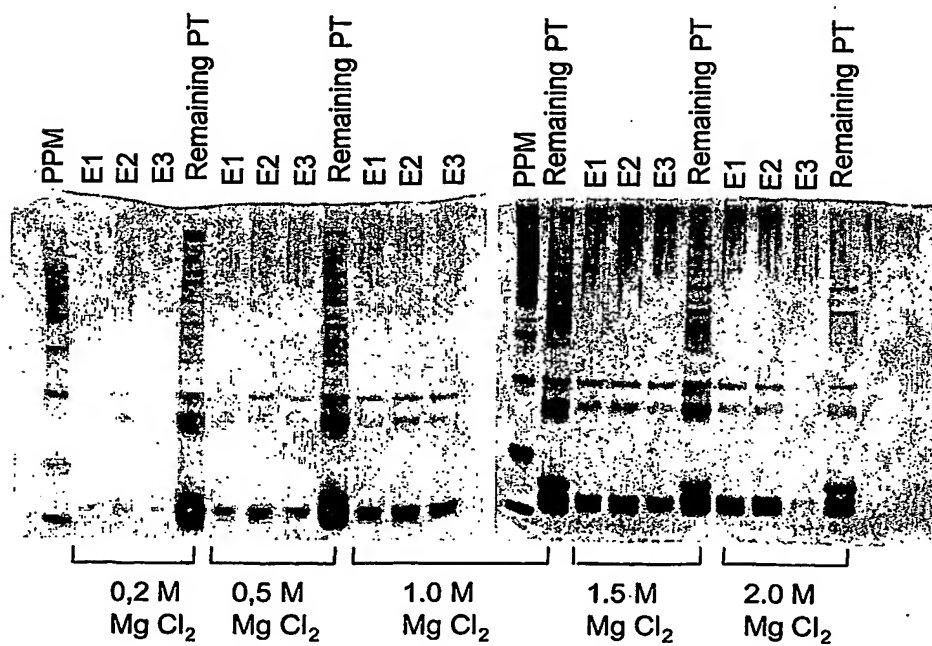


FIGURE 19

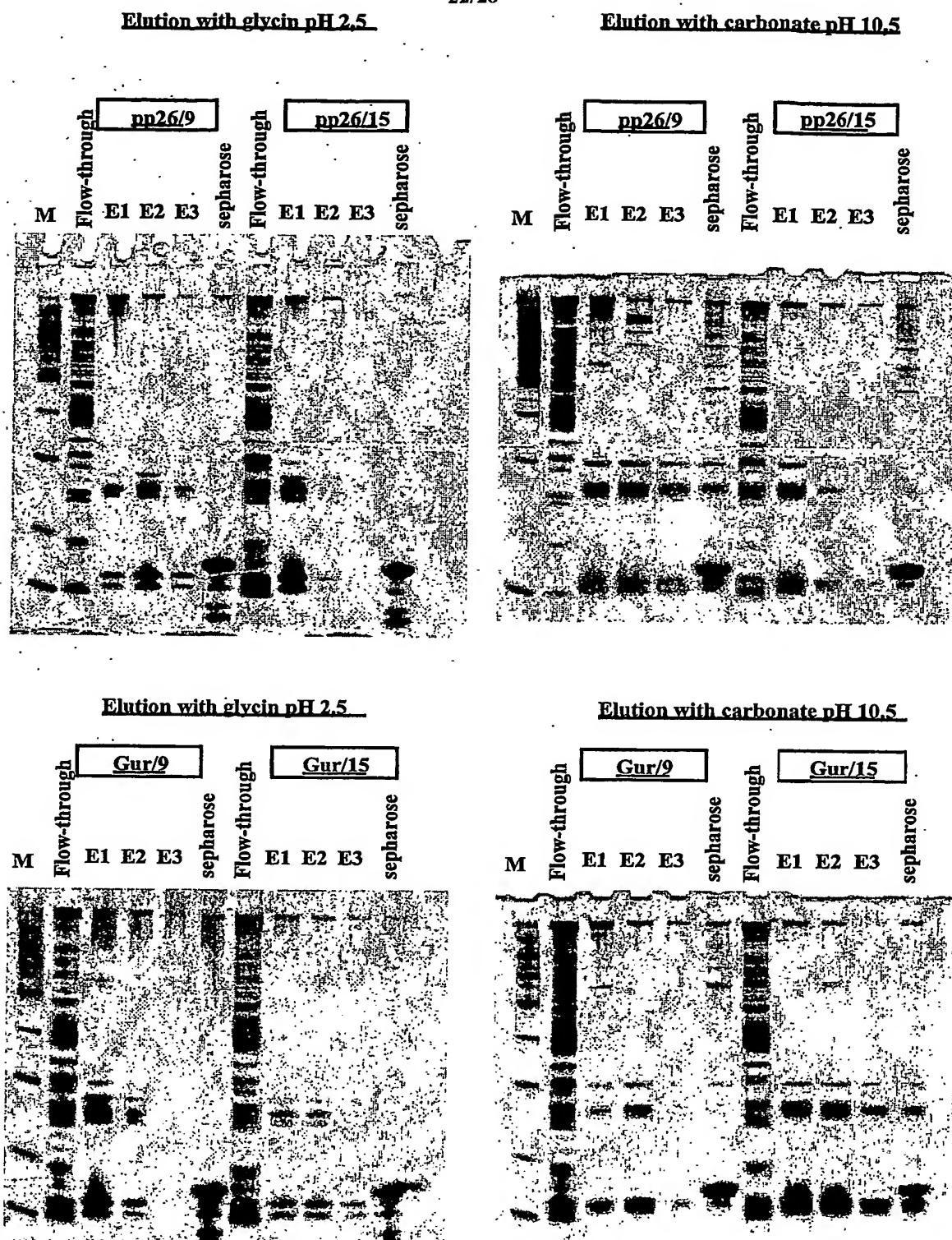
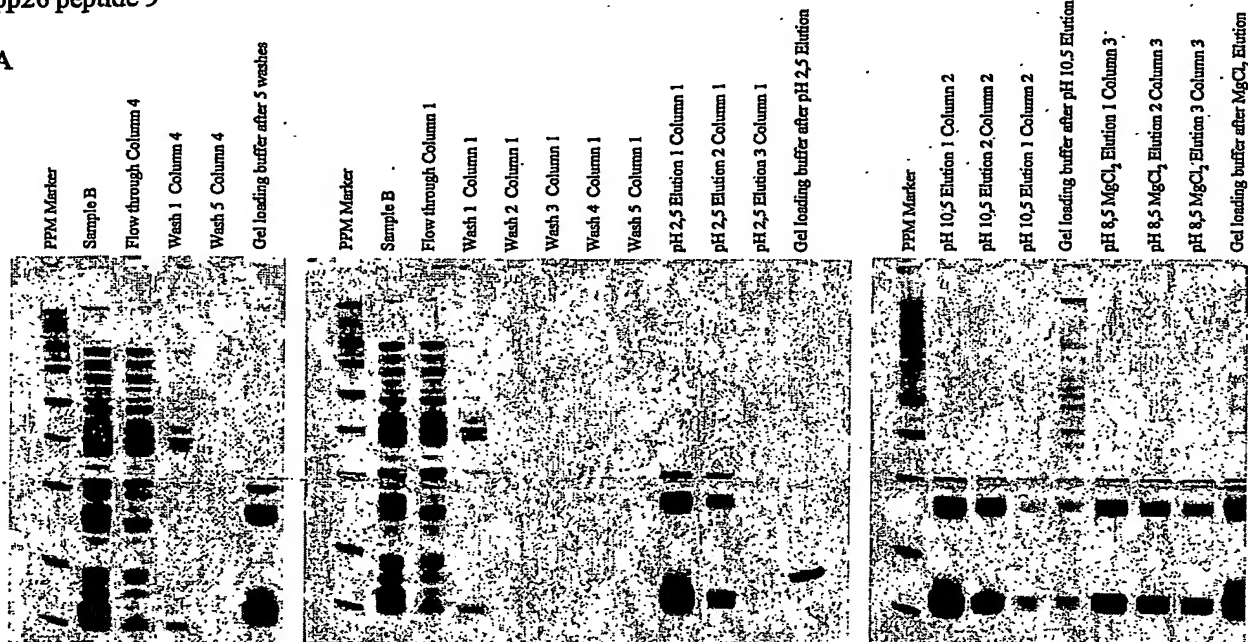


FIGURE 20

pp26 peptide 9

A



B

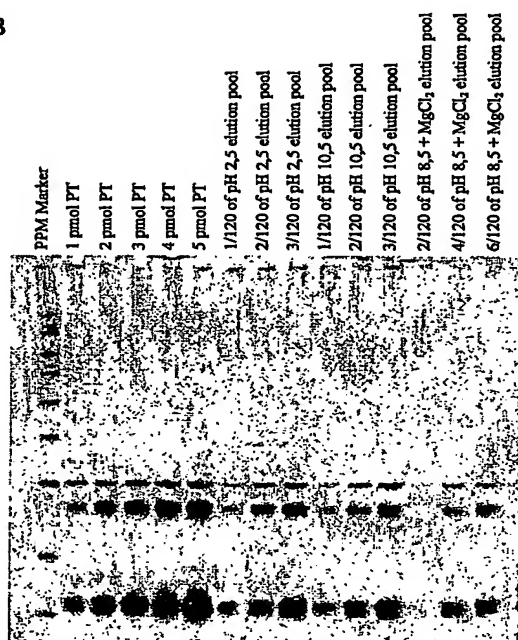


FIGURE 21

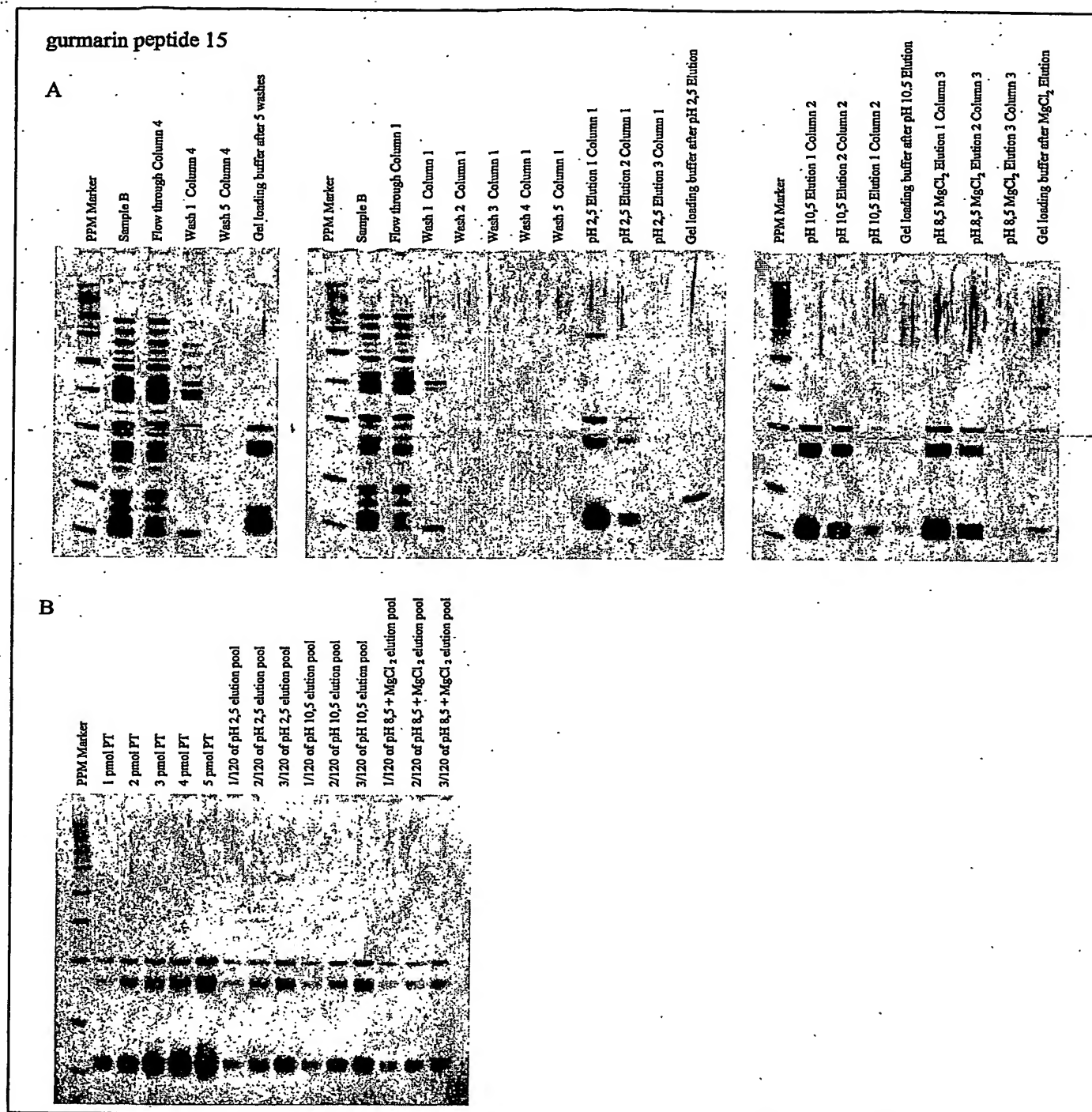
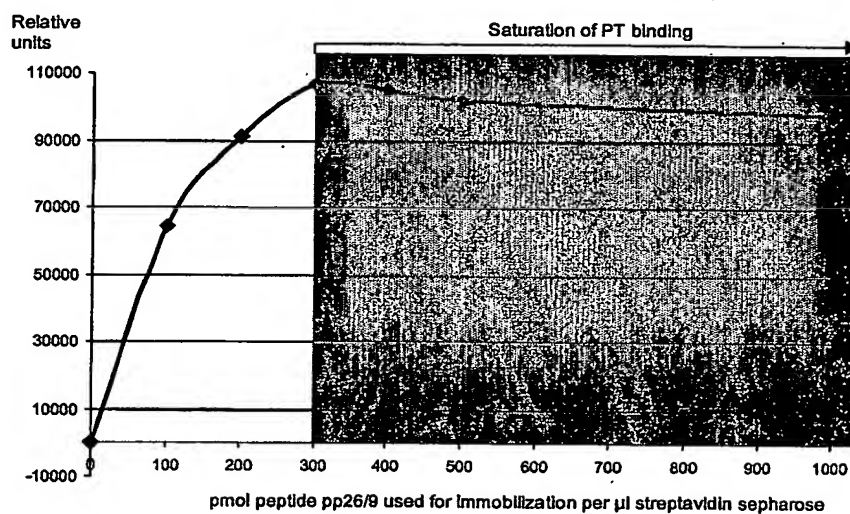
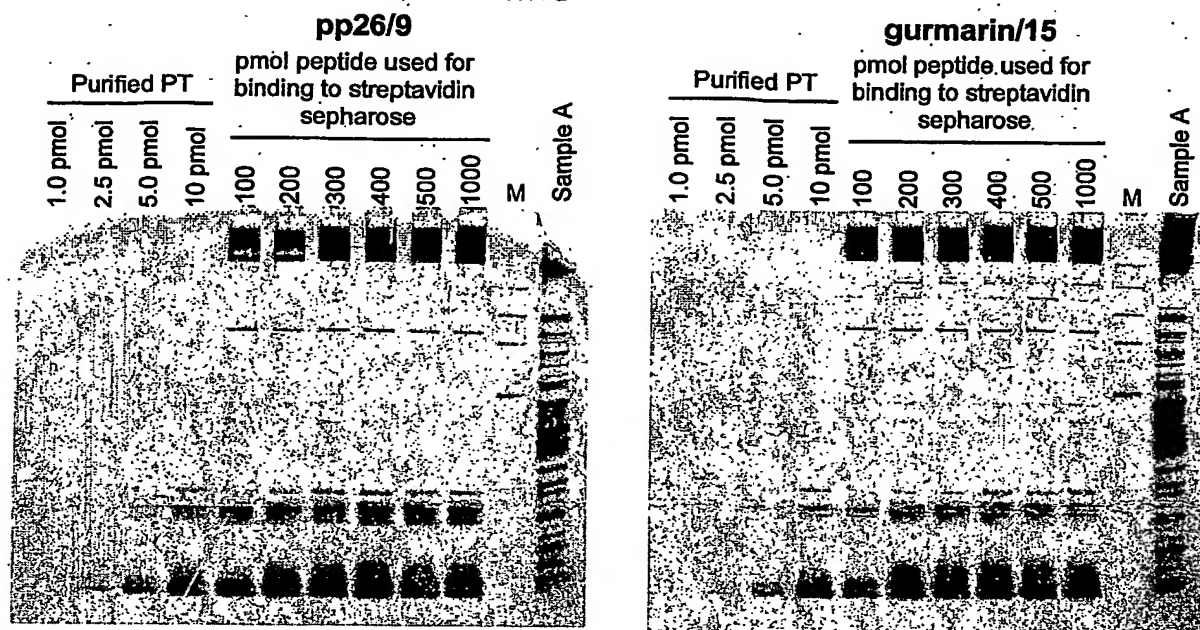
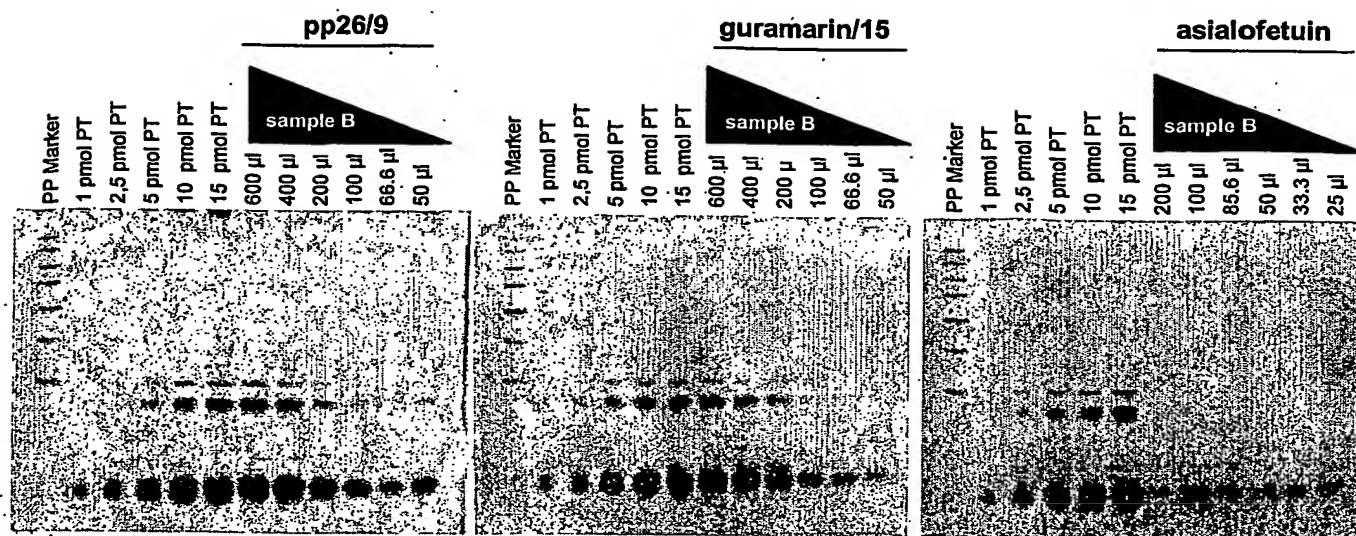


FIGURE 22

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FIGURE 23

**FIGURE 24**

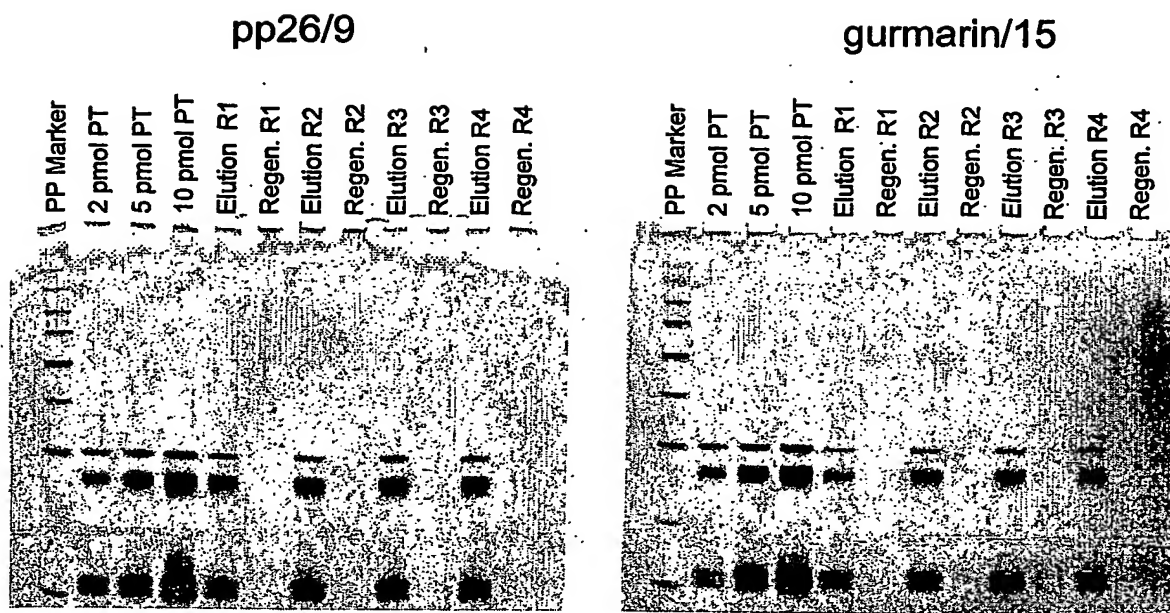


FIGURE 25

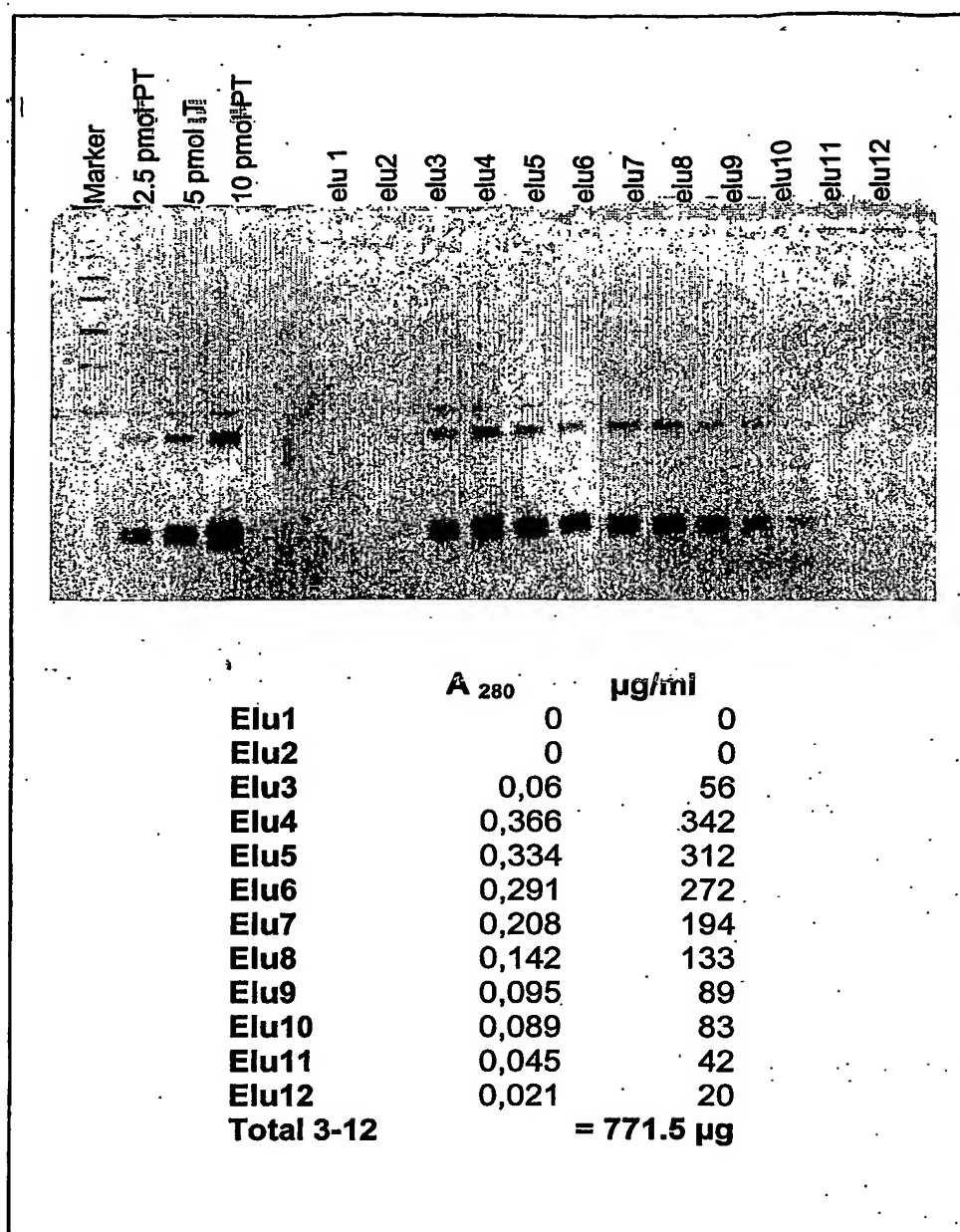


FIGURE 26

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